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Identification of a New Gene (*secA*) and Gene Product Involved in the Secretion of Envelope Proteins in *Escherichia coli*

DONALD B. OLIVER* AND JON BECKWITH

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115

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We have constructed lambda specialized transducing phages which carry an *Escherichia coli* gene (*secA*) involved in the secretion of certain envelope proteins. These phage have been used to show that *secA* is a new gene to the clockwise side of *envA*. The *secA* mutation previously described, *secA51*(Ts) (D. B. Oliver and J. Beckwith, Cell **25**:765–772, 1981), is recessive to the wild-type allele. We have also isolated Tn*5* insertions in the gene carried on the transducing phage to further define the gene. These phage were used to infect UV-irradiated cells to allow the identification of the *secA* gene product as a 92-kilodalton polypeptide and to show that transcription of *secA* is clockwise relative to the *E. coli* genetic map.

We have described a conditional lethal (temperature-sensitive) *Escherichia coli* mutant which is pleiotropically defective in the secretion of certain envelope proteins (11). This mutation maps in a new gene which lies at approximately 2.5 min on the bacterial chromosome and which we term *secA*. At high temperatures (37 or 42°C) the *secA* mutant strain accumulates cytoplasmic precursors of the maltose binding protein and a number of other envelope proteins. However, certain proteins still seem to be secreted normally. Sixteen additional temperature-sensitive mutants of this class map in this same gene (D. Oliver, M. Quinlan, and J. Beckwith, unpublished data). We have suggested that the *secA* mutations alter a component of the cell's secretion machinery so as to block normal protein export. If this explanation is correct, the characterization of the *secA* gene product and its function should contribute to an understanding of the secretion process.

In this paper we report a detailed analysis of the *secA* gene, including its precise location and orientation on the *E. coli* chromosome. We further show that the previously described *secA* mutation *secA51*(Ts) (11) is recessive to the wild-type allele. Last, we have used translational mapping to identify the *secA* gene product as a 92-kilodalton (92K) polypeptide.

MATERIALS AND METHODS

Media. Media for growth and plating of bacteria have been described (4, 9).

Phage strains. λ16-2 and λ16-25 carrying the *ftsA-envA* region were obtained from J. Lutkenhaus (6). The *Eco*RI cloning vector λ616 (10) was obtained from H. Revel.

Bacterial strains. Genetic nomenclature is from Bachmann and Low (1). *E. coli* K-12 MC4100 (F⁻ *araD* Δ*lacU169 relA rpsL*) was used for growth and plating of the λ phage. MM52 [MC4100, *secA51*(Ts)] was used as the standard *secA* mutant (11). AB2463/KLF4 (*thi-1 thr-1 leu-6 argE3 his-4 proA2 recA13 mtl-1 xyl-5 ara-14 galK2 lacY1 str-31 tsx-33 supE44*/F'104) was obtained from A. Wright. This strain, MM59 [MC4100, *secA51*(Ts) *recA1 srl*::Tn*10* φ (*malE-lacZ*)*72-47*(Hyb)], and MM60 (MC4100, *leu*::Tn*10*) were used in the diploid analysis. MM61 (MC4100, *leu*::Tn*10 ftsA12*), MM62 (MC4100, *leu*::Tn*10 ftsZ84*), and MM63 (MC4100, *leu*::Tn*10 envA*) were used in the complementation experiments. *E. coli* K-12 I7023 [F⁻ Δ*lacU169* Δ(*gal-bio*) *rpsL*] lacking the primary λ attachment site was used to obtain lysogens of λ16-25 integrated into the *ftsA-envA* region. I10.00 [F⁻ *araD* Δ(*lac-pro*) *metB argE*(Am) Nal^r *Rif^r su6* (P2)] was used to select for λ *spi*⁻ phage (12). Strain 294 (*endoI*⁻, *hsdR*⁻ *hsdM*⁺ *su*II) was used for transfection. MM64 (MC4100, *leu*::Tn*5*) was used to obtain TN5 insertions into the *secA* transducing phage. The bacterial strain used to examine phage-directed protein synthesis after UV irradiation was *E. coli* 159 (*uvrA gal rpsL*) and was obtained from J. Lutkenhaus.

Diploid analysis. The KLF4 episome was transferred from AB2463/KLF4 to MM59 by selecting for growth on arabinose minimal agar containing 10 μg of tetracycline per ml at 30°C. Several independent exconjugates were purified and tested for temperature sensitivity of growth at 42°C. None were temperature sensitive. These were then tested to ensure that the *secA51*(Ts) allele was still present by growing P1 *vir* on them, transducing MM60 to *leu*$^+$, and testing for the introduction of the *secA51*(Ts) allele.

Isolation of λDO2. Strain I7023 was lysogenized with λ16-25, and the lysogen was induced with UV irradia-

tion. *spi*⁻ phage were obtained by plating 0.1 ml of the lysate on I10.00. The 20 plaques which appeared were pooled, and a plate lysate was made with this same host. This plate lysate showed the presence of *secA* transducing phage as judged by spot tests on an MM52 lawn at 42°C. Therefore, a culture of MM52 was mixed with this lysate (multiplicity of infection = 0.2) and plated at 42°C for temperature-resistant transductants, which appeared at a frequency of approximately 10^{-3}. Three of these transductants were picked, purified, and induced for phage by UV irradiation. All three isolates yielded high-frequency transducing lysates for *secA*. One phage isolate, λDO2, was picked for further study.

Isolation of Tn5 insertions into *secA*. The procedure for obtaining TN5 insertions into λDO20 was essentially as described by Berg et al. (2). This Tn5 mutagenized phage stock was used to transduce MM52 to kanamycin resistance. Twelve of 600 Kan^r transductants tested were temperature sensitive for growth, indicating inactivation of the *secA* gene carried by the phage. Transducing phage from seven of these were chosen for further study and are designated λDO20(*secA1*::Tn5) through λDO20 (*secA7*::Tn5).

Preparation of phage and phage DNA. Phage were grown and purified and phage DNA was extracted essentially as described by Murray et al. (10).

Restriction and ligation conditions. The restriction and ligation conditions for phage DNA were similar to those described by Murray et al. (10).

Transfection. Strain 294 was treated with 50 mM $CaCl_2$ and transfected with 100 to 500 ng of ligated DNA according to the method of Mandel and Higa (8). The transfection mixture was plated in H top agar containing 0.02 ml of isopropylthio-β-D-galactoside (20 mg/ml) and 0.05 ml of 5-bromo-4-chloro-3-indolyl-β-D-galactoside (20 mg/ml).

Toothpick assay for *secA* transduction. The bacterial lawn on a plaque-containing plate was killed by chloroform vapors for 20 min. Individual plaques were stabbed with a toothpick, which was subsequently touched to a plate overlaid with 0.1 ml of an overnight culture of MM52 in top agar. After overnight incubation at 42°C, *secA* transducing phage gave a region of dense bacterial growth in the weakly growing surrounding lawn, whereas nontransducing phage gave only faint plaques.

Phage-directed protein synthesis after UV irradiation. Phage-directed protein synthesis after UV irradiation of the bacterial host was performed essentially as described by Lutkenhaus and Wu (7).

RESULTS

Isolation of *secA* transducing phages. Previous genetic analysis showed that the *secA* mutation *secA51*(Ts) is located at min 2.5 on the *E. coli* map (11) in or very near a cluster of genes concerned with cell envelope biogenesis (*ftsA ftsZ envA* [6, 7]). The *secA51* allele is 98% cotransducible with an *envA* allele. We obtained two *envA* specialized transducing phage, λ16-2 and λ16-25, to determine whether they also carry the *secA* gene. Neither of these phage complemented the *secA* mutant at 42°C, although both allowed for the formation of wild-type *secA51*$^+$ recombinants at a frequency of 10^{-4} (Table 1, lines 1 and 2). Two interpretations were possible: either the two phage do carry an intact *secA* gene but the *secA51* chromosomal allele is dominant, or the phage carry only a portion of the *secA* gene. To distinguish between these possibilities, we performed dominance tests on a merodiploid strain.

A *recA* strain containing *secA51* on the chromosome and *secA51*$^+$ on the KLF4 episome, MM59/KLF4, is not temperature sensitive for growth, showing that the *secA51* allele is indeed recessive (see Materials and Methods). The *secA51* allele is also recessive for the defect in maltose binding protein secretion (data not shown). Thus, since the two λ phages do not complement the *secA51* allele, they must carry only a portion of the *secA* gene.

To isolate a specialized transducing phage carrying the *secA* gene, we made use of the orientation of the bacterial genes carried by λ16-25. When λ16-25 integrates into Δ*att*λ host by bacterial DNA homology, the phage DNA is flanked by a duplication of the bacterial DNA (Fig. 1). By selecting for Spi⁻ phage which have lost a number of phage genes by an aberrant excision, given the DNA packaging constraints of λ, one also tends to select for phage which have picked up additional bacterial DNA to the clockwise side of *envA*. Thus, we were able to enrich for λ transducing phages carrying the *secA* gene by their Spi⁻ phenotype as well as by their ability to transduce the *secA* mutant to temperature resistance. The *secA* transducing

TABLE 1. Isolation of *secA* transducing phage[a]

Phage	*murC*	*ddl*	*ftsA*	*ftsZ*	*envA*	*secA*
λ16-2	C	C	C	C	C	R
λ16-25	—	—	C	C	C	R
λDO2	NT	NT	C	C	C	C
λDO11	NT	NT	—	—	—	C
λDO20	NT	NT	—	—	—	C

[a] To test for *ts*$^+$ transduction, 0.01 ml of a phage lysate was spotted onto a tryptone-yeast extract (TYE) plate previously spread with 0.1 ml of an overnight culture of the appropriate strain. Plates were scored after overnight incubation at 42°C. To test for *envA*$^+$ transduction, 0.01 ml of a phage lysate was mixed with 0.1 ml of a log-phase culture of MM63 grown in Luria broth (LB) plus 10 mM $MgSO_4$. After 20 min of adsorption at 37°C, the mixture was diluted 10-fold into LB and grown for 3 h at 37°C. A 0.01-ml portion of this mixture was spotted onto a TYE plate containing 2 μg of rifampin per ml. Plates were scored after overnight incubation at 37°C. Confluent growth in the region of the spot was scored as complementation (C), whereas the presence of single colonies was scored as recombinant (R). —, No complementation or recombination; NT, not tested. The results obtained for *murC* and *ddl* are from Lutkenhaus et al. (6).

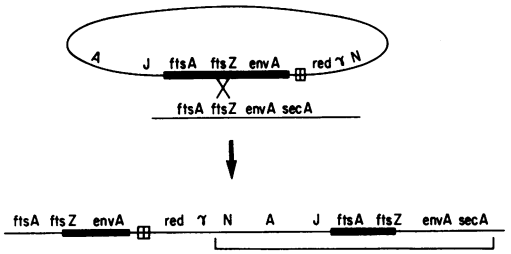

FIG. 1. Integration of λ16-25 carrying the *ftsA-envA* region into a homologous region of the chromosome of a host lacking the primary λ attachment site. The bracketed line below the integrated phage depicts a possible aberrant excision event in which the excising phage loses the *red* and γ genes while picking up additional genes to the right of *envA*, including the *secA* gene.

phage λDO2 isolated in this way (see Materials and Methods) carries not only the *ftsA*, *ftsZ*, and *envA* genes, as does its λ16-25 parent, but also the *secA* gene (Table 1, line 3). Restriction analysis of these two phage shows that λDO2 has picked up an additional 13 kilobases (kb) of bacterial DNA to the right of the bacterial insert present in λ16-25 (data not shown).

Since λDO2 carries so much bacterial DNA, we proceeded to construct a smaller *secA* transducing phage, using recombinant DNA techniques. λDO2 DNA was cleaved with *Eco*RI and mixed with the *Eco*RI-cleaved vector λ616. After ligation and transfection, individual plaques were tested for *secA* transducing ability (see Materials and Methods). One *secA* transducing phage obtained in this manner, λDO11, was chosen for further study. An *Eco*RI digest of λDO11 reveals that this phage is considerably smaller than λDO2 (Fig. 2, lanes 1 and 2). However, λDO11 carries at least three *Eco*RI fragments in addition to those comprising the phage vector (cf. lanes 2 and 6, Fig. 2). To determine the minimum number of *Eco*RI fragments needed for an intact *secA* gene, we repeated the cloning process, using λDO11. λDO11 DNA was cleaved with *Eco*RI, religated, and transfected for phage plaques. Three *secA* transducing phage, λDO20, λDO21, and λDO22, derived from λDO11 in this manner were chosen for further study. An *Eco*RI restriction analysis of these transducing phage (Fig. 2, lanes 3, 4, and 5) shows that, by the second round of restriction and selection for *secA* transducing phage, all three phages obtained gave identical restriction patterns. The *secA* gene must be coded for by portions of two *Eco*RI fragments which are 2.8 and 0.8 kb in size.

We have selected for Tn*5* insertions which inactivate the *secA* gene carried on λDO20 to further locate the *secA* gene on these two re-

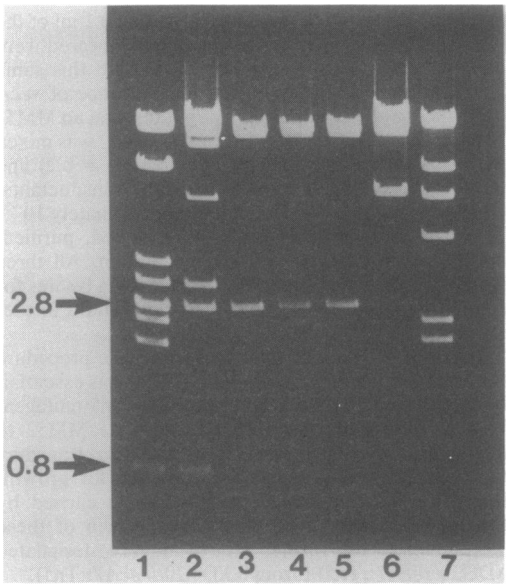

FIG. 2. A 2.5-μg portion of phage DNA in 0.05 ml of 10 mM Tris (pH 7.5)–150 mM NaCl–10 mM $MgCl_2$–1 mM dithiothreitol–100 μg of gelatin per ml was digested with 5 U of *Eco*RI at 37°C for 1 h. A 0.02-ml amount of the reaction mix was loaded onto a 0.8% horizontal agarose gel made with 40 mM Tris–20 mM sodium acetate–1 mM EDTA (pH 8)–1 μg of ethidium bromide per ml and electrophoresed at 150 mA for 2.5 h. The gel was photographed under UV light through a red filter. *Eco*RI digests of λDO2 (lane 1); λDO11 (lane 2); λDO20 (lane 3); λDO21 (lane 4); λDO22 (lane 5); λ616 (lane 6). A standard of λ cleaved with *Hin*dIII is included (lane 7).

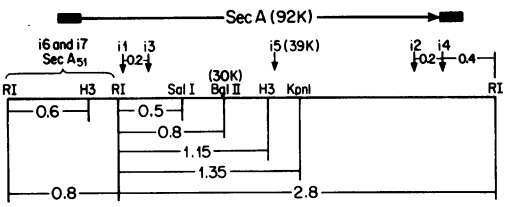

FIG. 3. Genetic organization of the *secA* gene. A restriction map of the region is given. *Eco*RI (RI), *Hin*dIII (H3), *Sal*I (SalI), *Bgl*II (BglII), and *Kpn*I (KpnI) restriction sites are shown, as well as the size of the restriction fragments (in kilobases) below the map. The positions of seven Tn*5* insertions (i1 to i7) are given above the map and were determined by using the restriction enzymes shown in the figure. The location of the *secA51*(Ts) mutation is also given. The approximate location of the *secA* gene coding sequences and the transcriptional direction of the gene are given at the top of the figure. This was possible by using the data given here as well as the translation mapping data presented in Fig. 4. The size of a truncated polypeptide (39K) synthesized by λDO20 (*secA5*::Tn*5*) and the portion of the *secA* gene (30K) included on a *secA-lacZ* fusion constructed in vitro by using the *Bgl*II site in *secA* are shown.

striction fragments. The Tn5 insertions can be easily mapped by restriction enzyme analysis (5). Of seven independent insertions analyzed, two mapped within the 0.8-kb *Eco*RI fragment and five mapped within the 2.8-kb *Eco*RI fragment. The five insertions within the larger restriction fragment map at positions covering most of this fragment (Fig. 3, insertions 1 to 5). This indicates that either the *secA* gene coding sequences occupy most of this DNA fragment (i.e., it is a large gene) or expression of the gene is abolished by insertions outside *secA* coding sequences (e.g., by polarity of Tn5 insertions within a multigene operon, or insertion within a positive regulator of *secA*). To discriminate between these two possibilities, translational mapping experiments were performed.

UV-irradiated cells were infected with λDO20 and its Tn5-containing derivatives to examine phage-directed protein synthesis. Comparison of the phage-directed proteins synthesized by λDO20 and its λ616 parent shows that only λDO20 synthesizes a rather large and prominent polypeptide of approximately 92K (Fig. 4, lanes 2 and 3). The additional two protein species synthesized by λ616, one of which is presumably β-galactosidase, are synthesized from the large *Eco*RI insert present in the λ616 displacement vector (10). Synthesis of the 92K polypeptide is abolished in UV-irradiated cells infected with phage λDO20 (*secA1*::Tn5) and λDO20 (*secA6*::Tn5) (Fig. 4, lanes 4 and 11). These phage contain Tn5 insertions within either the small *Eco*RI fragment or the proximal portion of the large *Eco*RI fragment (i.e., the insertion is immediately adjacent to the small fragment; see

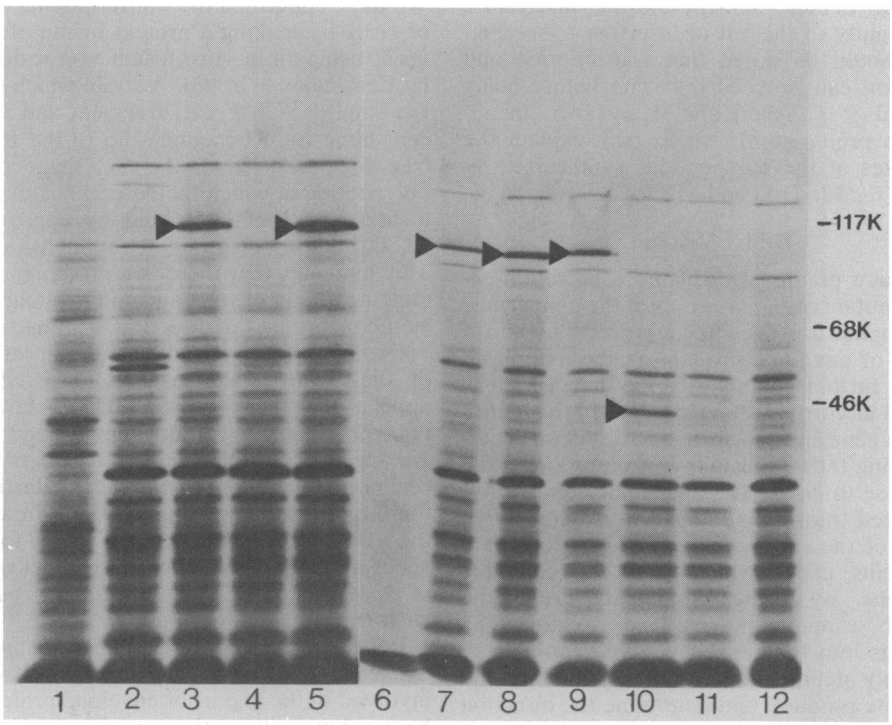

FIG. 4. Strain 159 was grown in M63 media containing 0.2% maltose until mid-logarithmic phase, when it was concentrated to approximately 2×10^9 cells per ml in 0.1 M $MgSO_4$. The cells were irradiated with a germicidal UV light at a distance of 66 cm for approximately 7 min. Phage were adsorbed to 0.1-ml samples of cells (multiplicity of infection = 10) at 37°C for 5 min, when 0.2 ml of prewarmed M63 medium containing 0.2% maltose was added. Five minutes later, 10 μCi of [^{35}S]methionine was added, and incubation was continued for an additional 5 min at 37°C. The phage-infected cells were then chilled on ice, sedimented for 5 min in an Eppendorf centrifuge, washed once, and resuspended in 0.1 ml of 125 mM Tris (pH 6.8)–2% sodium dodecyl sulfate–15% glycerol–5% β-mercaptoethanol. Samples, 0.025 ml, were incubated for 2 min at 100°C and loaded onto a 10% polyacrylamide gel. The gel was run at 35-mA constant current until the tracking dye reached the bottom. The gel was fixed and dried before autoradiography. The labeled protein profile of UV-irradiated 159 cells are given (lanes 1 and 6). Cells infected with λ616 (lane 2), λDO20 (lanes 3 and 7), λDO20(*secA1*::Tn5) (lanes 4 and 12), λDO20(*secA2*::Tn5) (lanes 5 and 8), λDO20(*secA4*::Tn5) (lane 9), λDO20(*secA5*::Tn5) (lane 10), and DO20(*secA6*::Tn5) (lane 11) are also given. The molecular weight standards are: β-galactosidase, 117,000; bovine serum albumin, 68,000; ovalbumin, 46,000.

Fig. 3). This result identifies the 92K polypeptide as the presumptive *secA* gene product (gp *secA*).

Infection with the other Tn*5*-containing phage gave a protein pattern consistent with all insertions being within the *secA* gene and the gene being transcribed left to right (Fig. 3). The λDO20 (*secA5*::Tn*5*) phage, whose insertion maps toward the middle of the large *Eco*RI fragment, synthesizes a truncated polypeptide of approximately 39K (Fig. 4, lane 10). λDO20 (*secA2*::Tn*5*) and λDO20 (*secA4*::Tn*5*) synthesize polypeptides of similar size to gp *secA* (cf. Fig. 4, lanes 7, 8, and 9). The polypeptide from λDO20 (*secA2*::Tn*5*) appears to be slightly smaller than gp *secA*, whereas the λDO20 (*secA4*::Tn*5*)-coded polypeptide is of similar molecular weight. This is compatible with the fact that both insertions map in the distal end of the 2.8-kb *Eco*RI fragment and that insertion 2 maps slightly to the left of insertion 4 (see Fig. 3). It should be noted that transcription and translation can proceed into Tn*5* before being abolished (R. R. Isberg and M. Syvanen, manuscript in preparation), which may explain the large sizes of the polypeptides synthesized by λDO20 (*secA2*::Tn*5*) and λDO20 (*secA4*::Tn*5*).

DISCUSSION

We have previously isolated a secretion-defective mutant of *E. coli* which at the nonpermissive temperature accumulates precursors to a number of envelope proteins in the cytoplasm (11). To further characterize the defect both in vivo and in vitro, we have proceeded to identify the *secA* gene and gene product. Standard genetic mapping techniques allowed us to locate the gene close to *envA*. The availability of an *envA* specialized transducing phage which contains a portion of the *secA* gene enabled us to isolate transducing phage which carry the complete *secA* gene. By further cloning the *secA* gene down to a minimum size and isolating Tn*5* insertions into the gene, we have been able to accurately define the *secA* gene, identify the *secA* gene product, and determine the direction of transcription of the gene.

secA is a new and rather large gene to the clockwise side of *envA*. The *envA* gene is contained on a 2.5-kb *Eco*RI fragment (7). *secA* is contained on two *Eco*RI fragments, 0.8 and 2.8 kb. The order of these fragments must be 2.5, 0.8, and 2.8 kb. This is the case since λ16-2 and λ16-25 both carry the 2.5-kb fragment and a portion of the 0.8-kb fragment (up to the *Hin*dIII site; see Fig. 3). This portion of the 0.8-kb fragment contains the portion of the *secA* gene corresponding to the *secA51* allele originally described (11). Furthermore, we have verified that λ16-25 specifically hydridizes only to the correct portion of the 0.8-kb fragment by Southern analysis (unpublished data).

The *secA* gene product is a polypeptide of approximately 92K. Tn*5* insertions in the very beginning of the gene result in the synthesis of no detectable gene product even in 15% polyacrylamide gels (unpublished data). A Tn*5* insertion toward the middle of the gene results in the synthesis of a truncated 39K polypeptide. Insertions at the end of the gene give rise to polypeptides of similar size to gp *secA*. These results are compatible with the observation that the former two classes of insertions fail to complement the *secA* temperature-sensitive mutant MM52 in diploid analysis, whereas the third class appears to give partial complementation (unpublished data).

The *secA* gene is transcribed in a clockwise direction, opposite to that inferred for *envA* (7). We have confirmed the transcriptional direction of *secA* by making a protein fusion of *secA* to *lacZ*, using an in vitro fusion vector developed by Casadaban et al. (3). A strain which contains the small 0.8-kb *Eco*RI fragment and the adjacent large *Eco*RI fragment up to the *Bgl*II site (see Fig. 3) fused to the *lacZ* gene makes a polypeptide in which the N terminus of β-galactosidase has been replaced by approximately 30,000 daltons of gp *secA* (unpublished data). This indicates that there is a promoter immediately upstream of *secA* and confirms our conclusion concerning the direction of transcription.

Combining our restriction and translation map of *secA* with a similar map for *envA* (7), it appears that there is enough DNA between the two genes to code for an average-sized protein. An appropriate transducing phage carrying this region intact could be constructed starting with the large transducing phage λDO2. In addition, the λDO2 transducing phage is useful for analysis of mutants which map to the clockwise side of *envA*, since this phage must carry roughly 10 such genes.

Results presented in this paper document the existence of a new gene in *E. coli* which is involved in the export of envelope proteins. We have shown that the previously described *secA*(Ts) allele (11) is recessive for both secretion and growth defects. This finding limits the number of models which can explain the nature of the secretion block in *secA* mutants. Models invoking the presence of an abnormal protein which somehow interferes with secretion by jamming up the membrane, for example, appear to be less likely. Location and quantitation of the *secA* gene product within the cell should further help to define its function.

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ADDENDUM IN PROOF

In further subcloning of the *secA* gene, we found that the position of one of the restriction sites given in Fig. 3 was incorrect. The *Hin*III site in the 2.8-kb *Eco*RI fragment should be just to the left of the *Bgl*II site shown in the figure.

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